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Abstract

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PI Name:	STRONGIN, ALEX Y
PI Email:	STRONGIN@BURNHAM.ORG
Project Title:	Screening Chemicals to Suppress MT1-MMP Synthesis in Cancer

Abstract: *DESCRIPTION (provided by applicant):* The purpose of this application is to describe our drug design effort to target the synthesis of MT1-MMP in cancer cells. MT1-MMP is a key player in cancer progression and metastasis. Insufficient knowledge of how MMPs including MT1-MMP work was the main reason for the failure of clinical trials in cancer. We have now identified previously uncharacterized, but highly important, additional functions of MT1-MMP. We discovered that MT1-MMP protects malignant cells against the host immunity. We also discovered that MT1- MMP is trafficked to centrosomes where this active protease cleaves integral centrosomal proteins and these events cause mitotic spindle aberrations and aneuploidy. Aneuploidy is a genetic marker of malignancy. In sum, these novel mechanisms allow neoplasms to survive immune attack, to invade the tissues, and to metastasize. We hypothesize that it is absolutely mandatory to suppress the protein synthesis of cellular MT1-MMP, as opposed to merely inhibiting its catalytic activity, in order to prevent malignant progression. This hypothesis warrants us to search for chemicals capable of suppressing the synthesis of MT1-MMP. Consistent with our hypothesis, we have already demonstrated that suppressing synthesis of MT1-MMP by siRNA significantly reduces the tumor growth of human fibrosarcoma HT1080 cells. Our specific aims are: (1) To identify low molecular weight chemicals capable of efficiently suppressing the MT1-MMP transcription. (2) To identify and to discard toxic compounds in an additional cell-based assay. We developed the cytoblotting assay employing HT1080 cells stably transfected with the luciferase reporter plasmid as the main tool for the screening of chemicals. This assay can be easily adapted to fit 384-well or 1536-well plates. This assay (Z'-factor = 0.8-0.9) is readily adaptable to automation. The coefficient of variation does not exceed 5%. Reproducibility between plates in day-to-day experiments also does not exceed 5%. The main technical parameters of the primary assay are as follows: assay volume, 0.1 ml; amount of cells per well, 10,000 in 96-well flat bottom plates; temperature, 37oC; assay time, 10 min. The reconstituted substrate stock is stable at -20'C for up to two weeks. The HT1080 cells stably transfected with the reporter plasmid and the detailed experimental protocol will be provided to the screening center. To discard toxic compounds, we will analyze viability of the cells co-incubated with the selected hits (50 fM) in an additional MTT-based assay.

We are convinced that our efforts will result in the identification of efficient drug-like suppressors of MT1-MMP synthesis. These compounds can then be used alone or in a combination with existing cancer therapies.

Thesaurus Terms: MT1-MMP, cancer, metastasis, centrosomal proteins, mitotic spindle aberrations, aneuploidy, tumor growth, human fibrosarcoma HT1080 cells, cytoblotting assay, HT1080 cells, luciferase reporter plasmid, 384-well plate format, 1536-well plate format,
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Institution:	BURNHAM INSTITUTE 10901 NORTH TORREY PINES ROAD LA JOLLA, CA 92037
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